In the Specification:

Please amend the Title at page 1, line 1 as follows:

~~Methods of Preventing Immune-Mediated Abortion by Inhibiting a CD-28 Mediated Costimulatory Signal~~ Prognostic and Diagnostic Methods for Spontaneous Abortion.

Please amend the "Related Applications" section on page 1 as follows:

This application is a divisional application of USSN 09/628,129, filed July 28, 2000, now U.S. Patent No. 6,613,327, which is a continuation-in-part application of USSN 09/362,812, entitled "Methods of Preventing Immune-Mediated Abortion By Inhibiting Costimulatory Signals", filed July 28, 1999, now abandoned, the contents of which are expressly incorporated by reference.

Please replace the paragraph at page 3, lines 7-26, with the following replacement paragraph:

Macrophages and NK cells are the predominant types of immune cells within the maternal decidua during early embryonic development while T cells accumulate during late ~~in~~ development. In a recent report, cultured human decidual cells were found to be capable of antigen presentation (Olivares *et al.*, 1997, *Biol Reprod* 57, 609-15). In an animal model of immune mediated spontaneous abortion, macrophage infiltration of the decidua is an early indication of immunological rejection and embryo resorption (Duclos *et al.*,1995, *Am J Reprod Immunol* 33, 354-66). It is generally thought that maternal/fetal cellular transfer is rare due to the chorionic ~~cells~~ cell layers that separate the two systems (Billington, 1992, *Baillieres Clin Obstet Gynaecol* 6, 417-38). However, evidence exists demonstrating that cross trafficking of cells between maternal and fetal systems occurs at very high frequency, albeit at very low levels. In a recent study in which mouse embryos were implanted into transgenic LacZ female mice, the

presence of maternal LacZ positive cells in the embryo proper was reported (Piotrowski and Croy, 1996, *Biol Reprod* 54, 1103-10). In a separate transplantation study, it was found that hypoimmunity of recipient mice toward MHC haplotype mismatched maternal skin grafts was correlated with the low level presence of maternal T cells in the neonate, presumably acquired in utero, present in the lymph nodes of the recipient mice (Zhang and Miller, 1993, *Transplantation* 56, 918-21). These observations suggest that low level cellular infiltration of the embryo by maternal lymphocytes is possible during the course of the pregnancy.

Please replace the paragraph at page 5, lines 13-16, with the following replacement paragraph:

Figure 1 shows a cytometric plot of antibody stained day 8.5 yolk sac cells. Panel A shows a two dimensional FACS plot of cells stained with anti-CTLA4 PE and anti-CD45 FITC antibodies. Panel B shows a two ~~Two~~ dimensional FACS plot of cells stained with anti-CTLA4 ~~anti-CTLA4~~ PE and anti-PECAM-1 FITC antibodies.

Please replace the paragraph at page 5, lines 30-34, with the following replacement paragraph:

Figure 4 shows effects of mCTLA4 Ig administration on the spontaneous abortion mouse model. CTLA4 treated pregnant mice from abortion prone matings (DBA x CBA) and non-abortion prone matings (BALC/c ~~Balb/C~~ x CBA) were sacrificed on day 12 of gestation and percent resorbed embryos were tabulated.

Please replace the paragraph at page 11, lines 20-31, with the following replacement paragraph:

Computer algorithms known in the art can be used to optimally align and compare two nucleotide or amino acid sequences to define the percent identity between the two sequences. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two

sequences is the algorithm of Karlin and Altschul (1990) Proc. Natl. Acad. Sci. USA 87:2264-68, modified as in Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-77. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, *et al.* (1990) J. Mol. Biol. 215:403-10. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) Nucleic Acids Research 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See ~~http://www.ncbi.nlm.nih.gov~~ web site of the National Center for Biotechnology Information.

Please replace the paragraph at page 12, lines 15-28, with the following replacement paragraph:

Specific residues of costimulatory molecules which are important in binding have also been determined. For example, the portion of CD28 which is critical for interaction with B7-1 and B7-2 has been determined using site directed mutagenesis, CD28 monoclonal antibody epitope mapping, receptor based adhesion assays, and direct binding of Ig-fusion proteins to cell surface receptors. A stretch of proline rich sequence in CD28, MYPPPY (SEQ ID NO: 1), has been found to be critical to the function of that protein (Truneh *et al.* 1996. *Mol. Immunol.* 33:321). Likewise, the regions of the B7-1 molecule which are important in mediating the functional interaction with CD28 and CTLA4 have been identified by mutation. Two hydrophobic residues in the V-like domain of B7-1, including the Y87 residue, which is conserved in all B7-1 and B7-2 molecules cloned from various species, were found to be critical (Fargeas *et al.* 1995. *J. Exp. Med.* 182:667). Using these, or similar, techniques, amino acid residues of the extracellular domains of costimulatory molecules which are critical and, therefore, not amenable to alteration can be determined.

Please replace the paragraph at page 15, lines 3-16, with the following replacement paragraph:

It will be appreciated by those skilled in the art that it is routine to generate antibodies to human costimulatory molecules by following standard techniques. Antibodies may either be polyclonal or monoclonal antibodies, or antigen binding fragments of such antibodies. Of particular significance for use in therapeutic applications are antibodies that inhibit binding of a costimulatory molecule with its natural ligand(s) on the surface of immune cells, thereby inhibiting costimulation of the immune cell. Preferred ~~a~~ costimulatory molecule antibodies are those capable of inhibiting or downregulating T cell mediated immune responses by binding B7-2, B7-1, or an ICOS ligand on the surface of a cell, *e.g.*, a B lymphocyte, and preventing interaction with CTLA4, CD28, and/or ICOS. Other preferred anti-costimulatory molecule antibodies are those which, in combination with a second antibody which binds to another costimulatory molecule, result in increased inhibition of costimulation of a T cell when compared to the first antibody alone, *e.g.*, a combination of anti-B7-1 and anti-B7-2 antibodies.

Please replace the paragraph at page 18, line 26 through page 19, line 2, with the following replacement paragraph:

B. Polyclonal Anti-Costimulatory Molecule Antibodies. Polycolonal antibodies to a purified costimulatory molecule protein or peptide having a costimulatory molecule activity can generally be raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of a costimulatory molecule immunogen, such as the extracellular domain of the ~~a~~ costimulatory molecule protein, and an adjuvant. For example, as described above, it may be useful to conjugate a costimulatory molecule (including fragments containing particular eptitope(s) of interest) to a protein that is immunogenic in the species to be immunized, *e.g.*, keyhole limpet hemocyanin, serum albumin.

Please replace the paragraph at page 21, lines 6-20, with the following replacement paragraph:

(b) A suspension of antibody-producing cells removed from each immunized mammal secreting the desired antibody is then prepared. After a sufficient time, the mouse is sacrificed

and somatic antibody-producing lymphocytes are obtained. Antibody-producing cells may be derived from the lymph nodes, spleens and peripheral blood of primed animals. Spleen cells are preferred, and can be mechanically separated into individual cells in a physiologically tolerable medium using methods well known in the art. Mouse lymphocytes give a higher percentage of stable fusions with the mouse myelomas described below. Rat, rabbit and frog somatic cells can also be used. The spleen cell chromosomes encoding desired immunoglobulins are immortalized by fusing the spleen cells with myeloma cells, generally in the presence of a fusing agent such as polyethylene glycol (PEG). Any of a number of myeloma cell lines may be used as a fusion partner according to standard techniques; for example, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209 ~~Rockville, Md~~.

Please replace the paragraph at page 22, lines 18-23, with the following replacement paragraph:

Media and animals useful for the preparation of these compositions are both well known in the art and commercially available and include synthetic culture media, inbred mice and the like. An exemplary synthetic medium is Dulbecco's minimal essential medium (DMEM; Dulbecco *et al.* (1959) *Virol* 8:396) supplemented with 4.5 gm/1 glucose, 20 mM glutamine, and 20% fetal ~~caf~~ calf serum. An exemplary inbred mouse strain is the BALB/c ~~Balb/c~~.

Please replace the paragraph at page 25, lines 1-23, with the following replacement paragraph:

The V-gene library cloned from the immunization-derived antibody repertoire can be expressed by a population of display packages, preferably derived from filamentous phage, to form an antibody display library. Ideally, the display package comprises a system that allows the sampling of very large variegated antibody display libraries, rapid sorting after each affinity separation round, and easy isolation of the antibody gene from purified display packages. In

addition to commercially available kits for generating phage display libraries (*e.g.*, the Pharmacia *Recombinant Phage Antibody System*, catalog no. 27-9400-01; and the Stratagene *SurfZAP*™ phage display kit, catalog no. 240612), examples of methods and reagents particularly amenable for use in generating a variegated anti-costimulatory molecule antibody display library can be found in, for example, ~~the~~ Ladner *et al.* U.S. Patent No. 5,223,409; ~~the~~ Kang *et al.* International Publication No. WO 92/18619; ~~the~~ Dower *et al.* International Publication No. WO 91/17271; ~~the~~ Winter *et al.* International Publication WO 92/20791; ~~the~~ Markland *et al.* International Publication No. WO 92/15679; ~~the~~ Breitling *et al.* International Publication WO 93/01288; ~~the~~ McCafferty *et al.* International Publication No. WO 92/01047; ~~the~~ Garrard *et al.* International Publication No. WO 92/09690; ~~the~~ Ladner *et al.* International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum Antibod Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J Mol Biol* 226:889-896; Clackson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *PNAS* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc Acid Res* 19:4133-4137; and Barbas *et al.* (1991) *PNAS* 88:7978-7982.

Please replace the paragraph at page 30, lines 28-34, with the following replacement paragraph:

CD28 stimulation has also been shown to result in protein tyrosine phosphorylation in a T cell (see *e.g.*, Vandenberghe, P. *et al.* (1992) *J. Exp. Med.* 175:951-960; Lu, Y. *et al.* (1992) *J. Immunol.* 149:24-29). Accordingly, in one embodiment, an agent which inhibits a costimulatory signal in a T cell inhibits tyrosine phosphorylation in the T cell. A preferred protein tyrosine kinase inhibitor is one which inhibits *src* protein tyrosine kinases. In one embodiment, the *src* protein tyrosine kinase inhibitor is herbimycin A, or a derivative or analogue thereof. Derivatives and analogues of herbimycin A include compounds which are structurally related to herbimycin A and retain the ability to inhibit the activity of protein tyrosine kinases. In another embodiment, the agent which inhibits protein tyrosine phosphorylation is a protein tyrosine phosphatase or an activator of a protein tyrosine phosphatase. By increasing the tyrosine

phosphatase activity in a T cell, the net amount of protein tyrosine phosphorylation is decreased. The protein tyrosine phosphatase can be a cellular protein tyrosine phosphatase within the T cell, such CD45 or Hcph. A cell surface tyrosine phosphatase on a T cell can be activated by contacting the T cell with a molecule which binds to the phosphatase ~~phosphatse~~ and stimulates its activity. For example, an antibody directed against CD45 can be used to stimulate tyrosine phosphatase activity in a T cell expressing CD45 on its surface. Accordingly, in one embodiment, the agent which inhibits protein tyrosine phosphorylation within the T cell is an anti-CD45 antibody, or a fragment thereof which retains the ability to stimulate the activity of CD45. Examples of antibody fragments include Fab and F(ab')2 fragments. Antibodies, or fragments thereof, can be provided in a stimulatory form, for example multimerized or immobilized etc.

Please replace the paragraph at page 34, line 26 through page 35, line 8, with the following replacement paragraph:

Downmodulation of immune responses can be demonstrated, *e.g.*, by a showing of a reduction in antibody titers in a subject to an embryo or paternal antigen, by a reduction in cellular reactivity against an embryo, *e.g.*, macrophage and/or NK cell infiltrate in the decidua. Additionally or alternatively a reduction in the numbers and/or activity of T cells specific for embryo and/or paternal antigens can be measured. Numbers of T cells specific for a given antigen can be determined, *e.g.*, using a standard limiting dilution assay. T cell activity can be determined, *e.g.*, by stimulating T cells from a subject *in vitro* with a embryo or paternal antigen (*e.g.*, using whole cells originating from the embryo or the father, using a preparation comprising a mixture of antigens or using a purified antigen preparation) in association with an MHC class II molecule, and a costimulatory signal, *e.g.*, provided by a stimulatory form of a B7 antigen, for instance a B7 antigen on a cell or by a soluble, stimulatory form of the peptide. Known cytokines released into the media can be identified, for example by measuring an increase in transcription of a cytokine gene, measuring proliferation and/or differentiation of cells that are responsive to a particular cytokine, or by a number of other methods using techniques that are well known in the art.

Please replace the paragraph at page 36, lines 5-11, with the following replacement paragraph:

Compositions which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams, films, or spray compositions containing such carriers as are known in the art to be appropriate. The carrier employed ~~in the~~ should be compatible with vaginal administration. Combinations can be, *e.g.*, in solid, semi-solid and liquid dosage forms, such as douches, foams, films, ointments, creams, balms, gels, salves, pastes, slurries, vaginal suppositories, or sexual lubricants.

Please replace the paragraph at page 39, lines 10-30, with the following replacement paragraph:

In one embodiment, the invention provides a method for detecting the presence of one or more of said genes in a biological sample. The method involves contacting the biological sample with an agent capable of detecting protein or nucleic acid molecules (*e.g.*, mRNA) such that the presence of one or ~~ore~~ more of said genes is detected in the biological sample. One agent for detecting mRNA is a labeled or labelable nucleic acid probe capable of hybridizing specifically to the mRNA of a particular gene. The nucleic acid probe can be, for example, the full-length cDNA, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and is sufficiently complimentary to specifically hybridize under stringent conditions to the particular mRNA. Probes can be designed using the publicly available sequences, determined by searching using the Genbank Accession Numbers: X53051 (human VCAM-1); NM_003005 (human P-selectin); NM_000450 (human E-selectin); HSIL05 (human IL-2 gene); X78437 (human IL-10); AF180562 and AF180563 (human IL-12); NM_000881 (human IL-11); U42625 (human TNFα); M15840 (human IL-1β); NM_003236 (human TGFα); M60315 (human TGFβ); L10918 (human RANTES); M54894 (human IL-6); J00219 (human IFN-γ); NM_005191 (human B7.1/CD80); U04343 (human B7.2/CD86); M35160 (human CD4); M36712 (human CD8); AF199028 (human GL50); or AF218312 (human ICOS). Sequences for

any of the aforementioned genes from species other than humans may be obtained by searching GenBank using the desired gene name and the name of the desired organism.

Please replace the paragraph at page 43, line 31 through page 44, line 16, with the following replacement paragraphs:

Developing mouse hematopoietic yolk sacs were examined for the presence of costimulatory receptors. Flow cytometric analysis revealed a $CTLA4^{+}$, PECAM-1^{+}, CD45- subpopulation of cells within dispersed yolk sac tissues as early as day 8.5 of embryonic development. Whole mount tissue immunohistochemistry localized CTLA4 staining to blood islands and vasculature of yolk sac cells, soluble B7-1 binding to yolk sac cells was inhibited by anti-CTLA4 antibodies, demonstrating the potential of yolk sac CTLA4 to recognize B7-1 as a cognate ligand. RT-PCR of mRNA prepared from yolk sac and maternal spleen yielded specific products when PCR primers specific for the extracellular domain of CTLA4 were used. Collagenase digestion of yolk sac tissues resulted in the elimination of anti-CTLA4 antibody staining, demonstrating yolk sac CTLA4 to be secreted protein associated with the extracellular matrix. To test whether a soluble/extracellular form of CTLA4 may have a role in maternal tolerance of the embryo, the effects of exogenously applied mCTLA4-Ig fusion protein were examined on an abortion prone mouse model (DBA x CBA). Administration of two 200 ug doses of CTLA4 Ig per mouse delivered intraperitoneally on days 4 and 6 of gestation revealed a reduction of spontaneous abortion from naturally occurring mean high levels (21%) to mean background levels (8%). These results indicate that yolk sac extracellular CTLA4 functions as an immunoprotective agent in tolerizing the maternal immune system against embryo during development, and suggests the possible use of soluble CTLA4 to ameliorate spontaneous abortions in humans.

Please replace the paragraph at page 45, lines 7-20, with the following replacement paragraph:

Tissue source/Mouse strains used. Mouse embryonic tissues used in this study were taken from timed pregnant strains CBA and Swiss Webster. No significant differences in CTLA4 surface phenotype profile were detected between yolk sacs of different mouse strains. Yolk sacs and embryos were removed from the uterus of sacrificed female mice between day 8.5 and day 14.5 post coitum. In spontaneous abortion mouse models, CBA female mice were allowed to copulate with either BALB/c ~~Balb/C~~ (control matings) or DBA (abortion prone) males overnight. The following morning, female mice with vaginal plugs (day 0.5 of gestation) were removed and housed apart from male mice. Two hundred □g of purified CTLA4 Ig were injected into each mouse interperitoneally ~~interperioneally~~ on days 4 and 6 of gestation corresponding to the time of implantation and the time when primary maternal immune responses initiate. On day 12 of gestation, mice were sacrificed and uterine horns removed for analysis. Percentage of resorbed embryos were quantified and recorded for each mouse examined.

Please replace the paragraph at page 45, line 22 through page 46, line 2, with the following replacement paragraph:

Flow cytometry. ~~Flow cytometry~~: Yolk sacs were dispersed mechanically by successive passage through 16, 18 and 20 gauge needles in the presence of 10% rabbit serum, used as an antibody blocking reagent. Dispersed yolk sac cells were filtered through nylon mesh to remove debris prior to cell staining. Directly conjugated antibodies (Pharmingen) used in this study are listed in Table 1. FITC-conjugated mouse B7-1-Ig fusion proteins were prepared at Genetics Institute. Propidium iodide was added prior to data acquisition for the fluorescent exclusion of dead cells. Data were collected on a Becton Dickenson FACScan flow cytometer using CellQuest software. For stringent analysis of collected data, the first 70 percentile peak of PI negative cells was gated for all plots presented in this study. In crossblocking experiments, yolk sac cells were preincubated with either 100 ug/ml unlabeled anti-CTLA4 antibody (Clone UC-4F10-11) or 100 μg/ml of control hamster Ig for 10 min. prior to the addition of 2 □g/ml B7-1-Ig FITC and incubation for 20 min. at 4 °C. Cells were washed in 2% fetal calf serum in PBS and stained with propidum iodide prior to FACS.

Please replace the paragraph at page 46, line 9 through page 47, line 2, with the following replacement paragraph:

RNA analysis. ~~RNA analysis~~. Total RNA was prepared from isolated tissues and cells using RNAStat-60 (Teltest B, Friendswood, TX). Poly-A RNA enrichment was performed using Promega PolyAttract System (Promega, Madison, WI) according to manufacture's protocol. To lessen the possibility of contaminating genomic DNA, RNA was treated with RQ-1 DNAse (Promega) prior to RT-PCR reactions. First strand synthesis and thermocycling conditions used were as suggested by the manufacturer (Gibco-BRL, Bethesda, MD). Reverse transcription was performed using oligo $dT_{(12)}$ primers for first strand extension and SuperScript reverse transcriptase in 40 ul reactions according to manufacturer's protocol. Completed first strand cDNA products were heat inactivated at 70 °C for 10 min and then stored at –20 °C. One microliter of first strand cDNA was used as amplification template for 100 □l PCR reactions of 50 pm each DNA primer (Table 2), in 50 mM KC1, 10mM Tris HC1, pH 9.0, 0.1% Triton X-100, 1.5 mM MgC12, 1 mM dNTPs and 2-5 units of Taq polymerase (Promega). Thermocycling reactions were performed with 42 cycles of 94°C, 1 min., 55°C 1 min, and 72 °C 1 min in a Robobcycler thermocycling device (Stratagene). Five microliters of completed PCR reactions were loaded onto 1.5% agarose mini-gels and electrophoresed. Amplification products were visualized by ethidium bromide staining.

Please replace the paragraph at page 47, line 31 through page 48, line 7, with the following replacement paragraph:

Example 3. Binding of yolk sac cells to B7-1 Ig fusion protein. To assess the potential of yolk sac cells in binding its cognate ligand in soluble form, yolk sac cells were stained with directly conjugated B7.1 Ig-FITC. When either yolk sac cells or control CHO mCTLA4-gpi cells were preincubated with 100 ug/ml control hamster Ig followed by staining with 2 ug/ml B7.1Ig-FITC ~~B7.1 IgFITC~~, positive cells were clearly detected in both cell types (Fig. 2). When yolk sac and CHO mCTLA4 gpi cells were preincubated with 100 ug/ml anti-CTLA4 antibody (clone UC-4F10-11) before the addition of 2 ug/ml B7.1Ig-FITC ~~B7-1 FITC~~, however, no positive staining

was observed for either cell type. These results demonstrate the functional ability of anti-CTLA4 blocking antibodies to compete with B7-1 binding to yolk sac cells, thus demonstrating yolk sac CTLA4 to be structurally functional in binding its cognate ligand in soluble form.

Please replace Table 2 on page 48 with the following replacement Table 2:

Name	Sequence
V1 (VL012)	247-CACAACACTGATGAGGTCCG-266 (SEQ ID NO: 2)
V2 (VL021)	257-TGAGTTCCAC CTTGCAGAGG-438 (SEQ ID NO: 3)
Actin1	105-GTCGTCGACA ACGGCTCCG GCATGTG-130 (SEQ ID NO: 4)
Actin2	357-CATTGTAGAAGGTGTGGTGCCAGAT-333 (SEQ ID NO: 5)

Please replace the paragraph at page 49, line 5 through page 50, line 2, with the following replacement paragraph:

Example 5. Association of yolk sac CTLA4 protein with the extracellular ~~extracelluar~~ matrix. In T lymphocytes, most CTLA4 is present intracellulary as reported. Therefore, the detection of a bright population of CTLA4 positive cells associated with the yolk sac was not expected. CLTA-4 or an alternate form of CTLA4 may exist as a secreted protein localized in the extracellular matrix (See, *e.g.*, GenBank Accession No: U90273, GenBank Accession No.: U90271; GenBank Accession No: U90270). To test the nature of the surface display on yolk sac CTLA4 positive cells, the effects of enzymatic removal of extracellular matrix prior to CTLA4 staining was examined. Mechanically dispersed day 12 yolk sacs and chemically dispersed CHO-CTLA4 gpi cells were incubated either in the presence or absence of collagenase, then stained with antibodies. In addition to staining for the presence of CTLA4, antibodies were used to detect CD44 antigen. CD44 is a broadly distributed extracellular matrix binding surface glycoprotein that is also found on embryonic tissues (Campbell *et al.*, 1995, *Hum Reprod* 10, 425-30; Lesley *et al.*, 1993, *Adv. Immnol.* 54, 271-335). CD44 exists in both soluble and

intrinsic membrane spanning surface forms (Rokhlin and Cohen, 1996, *Caner Lett* 107, 29-35; Yu and Toole, 1996, *J Biol Chem* 271, 20603-7). By flow cytomeric analysis, non-collagenase treated yolk sac cells revealed a population of $CD44^+$, $CTLA4^+$ double positive cells (Figure 3, R2) and a smaller population of single positive $CD44^+$, $CTLA4^-$ cells (Figure 3, R4). Staining of samples treated with collagenase prior to FACS resulted in the complete transformation of R2 double positive cells to double negative cells (Figure 3, R3) while the single positive $CD44^+$, $CTLA4^-$ population remained unaffected (Fig. 3). The preferential elimination of the double positive R2 population over that of the single positive R4 cell population is consistent with the model of extracellular CD44 and CTLA4 bound to ECM being preferentially removed by collagenase treatment. The presence of collagenase resistant $CD44^+$, $CTLA4^-$ cell population suggests that those cells display CD44 surface proteins and furthermore indicates the lack of nonspecific protease activity in this assay. In control experiments using CHO-CTLA4 gpi cells, collagenase treatment had no effect on CTLA4 surface staining (Fig 3), consistent with the absence of the requisite collagenase hydrolysis FALGPA motif in the adult murine CTLA4 extracelluar protein sequence.

Please replace the paragraph at page 50, lines 4-20, with the following replacement paragraph:

Example 6. Animal model of soluble CTLA4 function. The DBA x CBA abortion prone mouse model is the standard model for the study of immune mediated spontaneous abortion (Toder *et al.*, 1989, *J Reprod Fertil Suppl* 37, 79-84). In pregnant CBA female mice, the resorption rate of DBA:CBA hybrid embryos occur in 21-30% of the embryos compared with 8% resorption in control Balb C: CBA embryos. In this model system, a maternal immunological rejection response towards the embryo initiates at day 6 of gestation, 2 days following implantation (Duclos *et al.*, 1995, *Am J Reprod Immunol* 33, 354-66). To assess the potential of a soluble CTLA4 to suppress maternal rejection of the embryo, two 200 μg doses of CTLA4 Ig <u>were</u> ~~was~~ administered to pregnant mice with ~~high rates with~~ either high or low rates of spontaneous abortion. Doses were administered at days 4 and 6 of gestation, corresponding to the time of

implantation (day 4) and day when immunological reactions to the embryo is first detected (day 6). Quantitation of resorbed embryos was performed on day 12 of gestation. In highly aborting CBA x DBA mated mice (n = 14), embryonic resorption rates averaged of 8%, identical with that of control BALB/c ~~Balb/C~~ x DBA mated mice (n = 8), demonstrating the efficacy of CTLA4 Ig treatment to ameliorate spontaneous abortion in this animal model Fig 4.

Please replace the paragraph at page 51, line 27 through page 52, line 3, with the following replacement paragraph:

Duplicate samples were reverse transcribed for 30 minutes at 60°C, followed by 40 cycles of amplification for 15 seconds at 95°C and one minute at 60°C using the ABI Prism 7700 sequence detection system as described by the manufacturer (Perkin Elmer). Gene-specific amplification was detected as a fluorescent signal during the amplification cycle. Gene-specific message quantification was evaluated by fluorescence intensity levels of unknown samples compared to fluorescence intensity of known mRNA levels. Amplification of a house keeping gene, murine GAPDH, was performed on all samples to account for RNA level ~~levels~~ variations. All genes were normalized to GAPDH mRNA levels and levels of gene-specific messages were depicted as normalized Taqman units as determined by standard curve.

Please replace the paragraph at page 52, lines 4-25, with the following replacement paragraph:

In vivo Radiolabeled Adhesion Molecule Expression. Pregnant CBA/J mice were anesthetized at gestation day 12 with an intraperitoneal injection mixture of 100 µl each of ketamine (50 mg/ml) and xylazine (2.75 mg/ml). The left jugular vein and right carotid artery were cannulated with polyethylene tubing (PE-10). Radiolabeled MAb (^{131}I-nonbinding and ^{125}I –VCAM-1) ~~^{125}I–VCAM-1~~ preparation was injected into the mouse via the jugular vein catheter, followed by an additional 0.2 ml of normal saline to aspirate all of the MAb in the syringe and tubing. Syringe was saved in a separate tube for radioactive counts. Antibody was allowed to circulate *in vivo* for 5 minutes at which time two 0.2 ml samples of blood were collected via the carotid artery

catheter. Ten units of heparin (10 µl from a 1000 U/ml) were added to the blood sample and centrifuged at 3000g for 10 minutes and 50 µl of plasma was collected and placed in a in new tube for radioactive counts. Next, 40 units of heparin (40 µl of 1000 U/ml) were injected into the jugular vein catheter immediately following the second blood draw. Whole body perfusion was performed by simultaneous infusion of bicarbonate buffered saline (BBS) via the jugular vein catheter and blood removal from the carotid artery catheter; perfusion was performed until the effluent was clear (approximately 15-20 mls). Next, mice were backflushed with an infusion of 10-15 mls of BBS via the carotid artery catheter by severing the inferior vena cava at the thoracic level. Finally, designated tissues were dissected, dipped in distilled water, blotted dry, weighed, and placed in scintillation tubes for gamma counter for irradiation counts on dual isotope spectrum. Calculation of specific antibody incorporation was determined by the following equation:

Please replace the paragraph at page 53, lines 104, with the following replacement paragraph:

Example 8. Staining of Placental Sections. Hemotoxylin and eosin staining of normal and resorbed placentas revealed that in resorbed diseased placenta ~~plecenta~~, there was cellular infiltration of the maternal decidua basalis, and there was also loss of normal placental structure in the fetal aspects of the placenta.

Please replace the paragraph at page 53, line 41 through page 54, line 6, with the following replacement paragraph:

Example 10. *In vivo* accumulation of ^{125}I Labeled VCAM-1 MAb. *In vivo* accumulation of ^{125}I labeled VCAM-1 monoclonal antibody (MAb) in the two mating crosses did show organ-specific differences between spleen and kidney (Figure 5A), but did not show significant differences in VCAM-1 binding between the two mating groups. However, VCAM-1 binding was found to be elevated in the placentas of the DBA X CBA cross, when compared to the BALB/c ~~Balb/C~~ X

CBA mating combination (Figure 5A). Particularly, the highest accumulation was seen in the resorbing placentas of the pathologic mating group.

Please replace the paragraph at page 57, lines 1-21, with the following replacement paragraph:

Current models of maternal immunological tolerance of the embryo have centered on two complementary mechanisms: utilization of indirect cytokine and hormonal signaling and the active immunosuppression of maternal lymphocytes. Recently, a number of known immunomodulatory cytokines were described that have been demonstrated to influence maternal tolerance of the embryo. Whereas the Th1 cytokines promoting cellular cytotoxicity (*e.g.*, during an intracellular parasitic infection) such as TNF-α, IFN-γ, IL-2, IL-1, IL-6, IL-12, and RANTES have been demonstrated to increase spontaneous abortion rates in abortion prone mice, the presence of Th2 cytokines such as CSF-1, GM-CSF, IL-10, IL-4, IL-11, TGF and IL-3 which promote humoral immunity, have been shown to correlate with reduced abortion rates (Chaouat *et al.*, 1990, *J Reprod Fertil* 89, 447-58; Gafter *et al.*, 1997, *J Clin Immunol* 17, 408-19). Active immunosuppression has also been documented in studies with factors derived from embryonic sources. In the past, some unidentified proteins and anti-paternal blocking antibodies have been reported to confer modest immunosuppression to the embryo (Herrera-Gonzalez and Dresser, 1993, *Dev Comp Immunol* 17, 1-18). Examples 7-11 indicate that expression levels of numerous cytokines, as well as adhesion molecules and cell-surface antigens, are altered in mice during pathologic pregnancy, but that many can be normalized by treatment with CTLA4Ig. Altered expression levels of these genes, therefore may be used as diagnostic criteria for determining whether a subject is at risk <u>for,</u> ~~for~~ or developing or suffering <u>from,</u> ~~from~~ an immune-mediated spontaneous abortion.